IN THE CLAIMS:

Please cancel claims 18-24 without prejudice or disclaimer.

REMARKS

The Claims

Claims 18-24 have been canceled. Claims 25-27 are under consideration.

The Information Disclosure Statement

In the Office Action dated November 19, 2003, the Examiner states that reference AF in the IDS files July 30, 2001 [Oshima et al., 'Development of bioartificial livers from the tissue engineering viewpoints', "Artificial Organs (Jinkou Zouki)" (a journal published by Japanese Society of Artificial Organs) Vol 27, No. 5, p. 724-732, 1998] was not considered because its relevance was allegedly not indicated in the International Search Report. However, applicants respectfully point out that this reference was, indeed, referred to in the International Preliminary Examination Report (IPER) completed on January 12, 2001. A copy of the IPER, with the reference highlighted, is attached for the convenience of the Examiner, as is a copy of the article (in Japanese).

The Drawings

A Petition under 37 CFR 1.84(a)(2) for acceptance of the color photographs is attached to this Reply, as is the appropriate fee set forth under 37 CFR 1.17(h) and three sets of the color photographs. The first paragraph of the brief description has been amended as suggested by the Examiner.

The Enablement Rejection

(A) The Office Action states that claims 18-23 and 25-27 are rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. In

view of the cancellation of claims 18-23, the rejection of those claims is moot Claims 25-27 are enabled by the specification, for at least the following reasons. References referred to in this argument are attached for the convenience of the Examiner.

The specification of the present invention describes, "For examination of the cell differentiation or the stages, it is preferable to conduct quantitative analysis using many kinds of gene markers or antibodies simultaneously with observation of the tissues.", and clearly states that the stage of organ development can be determined by observation of tissues as well, and that "first carrying out extensive experimentation to determine the parameters using DNA as stage markers for practice of the invention" is not always needed.

Further, the specification of the present invention also describes, "More defined testing can be performed by using genomic DNA, which expresses corresponding to the stage of in vitro induced organ, as a molecular marker."

In addition, Professor Makoto Asashima of University of Tokyo, the inventor of this application and an authority of developmental biology, has declared in the Declaration as follows: "For practice of the invention, in case a particular organ in a particular vertebrate is targeted, the person skilled in the art can easily (without undue experimentation) determine which gene DNA can be used as stage markers, by ordinary methods such as the differential display method. For researchers in this field, the opinion 'As to all of amphibians, birds, bony fish, chimaera chondrichthian, mammal, reptile, etc., one skilled in the art could not practice the claimed invention without first carrying out all extensive experimentation.' is unacceptable."

As a matter of fact, stage marker gene DNAs for a number of animals and organs are known as described below, validating the content of the Declaration by the above-mentioned Professor Makoto Asashima, a person skilled in the art.

First, there is a database called the "Ontology of Human Developmental Anatomy", regarding the stage marker molecules of mammals such as mouse, rat, human, etc., on the web site of the University of Edinburgh. The database shows the stage markers of lung, mammary gland, pancreas, prostate gland and salivary gland, together with the information on the species, organ, stage and reference. The following tables

were obtained from the database mentioned above (http://www.ana.ed.ac.uk/anatomy/database/humat/).

1. Marker Molecules of Lung

Expression of Cytokines during Lung Development
(http://www.ana.ed.ac.uk/anatomy/database/lungbase/cytkintb.html)

Species and Organ	Molecule	Stage	Reference
Rat lung	act/inh beta-A	E8- E20	Roberts,VJ. 1994
Mouse lung	Amphiregulin	E12	Schuger,L. 1996
Mouse lung	Bmp-2 (mRNA)	E11.5	Bellusci,S. 1996
Mouse lung	Bmp-4 (mRNA)	E11.5	Bellusci,S. 1996 Urase,K. 1996
Mouse lung	Bmp-7 (mRNA)	E11.5	Bellusci,S. 1996 Lyons,KM. 1995
Human lung	EGF (mRNA)	Wk11- Wk41	Ruocco,S. 1996
Mouse lung	EGF	E11- E18	Warburton,D. 1992
Human lung	EGF	Wk11- Wk41	Ruocco,S. 1996
Mouse lung	Endothelin-1 (mRNA)	E17- adult	Maemura,K. 1996 Chan,TSK. 1995
Mouse lung	Endothelin-3 (mRNA)	E17- adult	Maemura,K. 1996 Chan,TSK. 1995
Rat lung	aFGF	E13- E20	Fu,Y-M. 1991
Human lung	bFGF (mRNA)	Wk12- Wk16	Gonzalez,AM. 1996
Human lung	bFGF	Wk12- Wk16	Gonzalez,AM. 1996 (Gonzalez,AM. 1990)
Mouse lung	GRP (mRNA)	E12- P14	King,KA. 1995
Rat lung	IGF-I (mRNA)	E15- E21	Retschbogart,GZ. 1996, Moatsstaats,BM. 1995, De-Chiara,TM. 1990
Rat lung	IGF-I	E16- E20	Klempt,M. 1992
Rat lung	IGF-II (mRNA)	E15- E21	Retschbogart,GZ. 1996, Moatsstaats,BM. 1995, De-Chiara,TM. 1990
Rat lung	IGF-II	E16- E20	Klempt,M. 1992
Rat lung	HGF (mRNA)	E14- adult	Shiratori,M. 1996
Rat lung	KGF (mRNA)	E14- adult	Shiratori,M. 1996 Dekowski,SA. 1996
Rat lung	KGF (mRNA)	E12 & E13	Post,M. 1996
Rat lung	KGF	E12 & E13	Post,M. 1996
Rat lung (Mouse lung)	PDGF-A (mRNA)	E12- E14	Souza,P. 1995 (Orr-Urtreger,A. 1992)
Rat lung	PDGF-AA	E12- E22	Han,RNN. 1992
Rat lung	PDGF-BB	E12- E22	Han,RNN. 1992
Mouse lung	PPET-1 (mRNA)	E11.5 E18.5	Chan,TSK. 1996
Mouse lung	Shh (mRNA)	E11.5	Bellusci,S. 1996 Urase,K. 1996
Human lung	TGF-alpha (mRNA)	wk11- wk41	Ruocco,S. 1996
Human lung	TGF-alpha	wk10- wk41	Ruocco,S. 1996 Strandjord,TP. 1993
Mouse lung	Pro- TGF beta-1	E11- E18	Heine,UI. 1990
Mouse lung	TGF beta-1 (mRNA)	E10.5 E16.5	Schmid,P. 1991
Mouse lung	TGF beta-1	E11- E18	Heine,UI. 1990 (Pelton,RW. 1991)
Mouse lung	TGF beta-2 (mRNA)	E9.5- E16.5	Schmid,P. 1991 Millan,FA. 1991
Human lung	TGF beta-2 (mRNA)	32dpc 71dpc	Gatherer,D. 1990
Mouse lung	TGF beta-2	E17.5 E18.5	Pelton,RW. 1991
Mouse lung	TGF beta-3 (mRNA)	E9.5-E16.5	Schmid,P. 1991 Millan,FA. 1991
Human lung	TGF beta-3 (mRNA)	32dpc	Gatherer,D. 1990
Mouse lung	TGF beta-3	E17.5 E18.5	Pelton,RW. 1991
Mouse lung	VEGF (mRNA)	E13- adult	Amin,SB. 1996

Mouse lung (Rat lung)	Wnt2 (mRNA)	E11.5- E18.5	Bellusci,S. 1996 Levay-Young,BK. 1992
Human lung	Wnt7A (mRNA)	Fetal	Ikegawa,S. 1996
Mouse lung	Wnt10b (mRNA)	E15.5	Wang,JW. 1996

Expression of Receptors and Signal Transduction Molecules during Lung Development (http://www.ana.ed.ac.uk/anatomy/database/lungbase/recepttb.html)

Species and Organ	Molecule	Stage	Reference
Rat lung	ActR-IIB (mRNA)	E8- E20	Roberts,VJ. 1994
Mouse lung	ALK3 (mRNA)	E9.5- E15.5	Dewulf,N. 1995
Mouse lung	ALK6 (mRNA)	E9.5- E15.5	Dewulf,N. 1995
Mouse lung	bek (mRNA)	E9.5- E16.5	Orr-Urtreger,A. 1993 Peters,KG. 1992
Mouse lung	BmpR typeI(mRNA)	E11.5	Bellusci,S. 1996
Rat lung	CD44 isoforms	E12- adult	Weber,B. 1996
Rat lung	CD44s	E12- adult	Weber,B. 1996
Rat lung	CD44 variant V6	E12- adult	Weber,B. 1996
Human lung	Dax-1 (mRNA)	Adult	Bae,DS. 1996
Mouse lung	EGF-R	E11- E18	Warburton,D. 1992
Mouse lung	EGF-R	E11.5+3dys	Volpe,MV. 1996
Human lung	EGF-R	Wk11- Wk41	Ruocco,S. 1996
Human lung	EMP-2(mRNA)	Adult Fetal	Taylor,V. 1996
Human lung	EMP-3(mRNA)	Adult Fetal	Taylor,V. 1996
Mouse lung	Fas (mRNA)	E16.5 E18.5	French,LE. 1996
Mouse lung	FGFR-1 (mRNA)	E9.5- E16.5	Peters,KG. 1992
Human lung	FGFR-1	Wk12-Wk16	Gonzalez,AM. 1996 (Partanen,J. 1991)
Human lung	FGFR-3 (mRNA)	Wk17-Wk18	Partanen,J. 1991
Human lung	FGFR-4 (mRNA)	Wk17-Wk18	Partanen,J. 1991
Mouse lung	flk1 (mRNA)	E13- adult	Amin,SB. 1996
Human lung	hGHR (mRNA)	11.5w-term	Zogopoulos,G. 1996
Human lung	hGHR	8.5-20wks	Simard,M. 1996
Mouse lung	GRP-R(mRNA)	E12- P14	King,KA. 1995
Rabbit lung	GRP-R(mRNA)	E20- E29	Wang,DS. 1996
Human lung	GRP-R(mRNA)	19wks	Wang,DS. 1996
Rat lung	IGF typeI-R (mRNA)	E16- E22	Moatsstaats,BM. 1995
Rat lung	IGF typeI-R (mRNA)	E15- E21	Retschbogart,GZ. 1996 Moatsstaats,BM. 1995
Rat lung	IGF (mRNA) typeII-R	E15- E21	Retschbogart,GZ. 1996
Rat lung	KGFR (mRNA)	E17- P10	Dekowski,SA. 1996
Rat lung	KGFR (mRNA)	E12 & E13	Post,M. 1996
Rat lung	KGFR	E12 & E13	Post,M. 1996
Rat lung	LAR-PTP2 (mRNA)	E14 - adult	Kim,H. 1996 Rotin,D. 1994
Rat lung	LAR-PTP2	E12.5- adult	Meneghetti,A. 1996
Mouse lung	MR (mRNA)	E9.5- P0	Brown,RW. 1996
Rat lung	MR (mRNA)	E18- E20	Catlin,EA. 1996
Rat lung □Mouse lung□	PDGFR-alpha (mRNA)	E12- E14	Souza,P. 1995 and 1996 (Orr-Urtreger,A. 1992)
Rat lung	PDGFR-beta (mRNA)	E13- E15	Souza,P. 1996
Human lung	PGT (mRNA)	Adult Fetal	Lu,R. 1996
Mouse lung	PRL-R (mRNA)	E12- P1	Brownborg,HM. 1996
Mouse lung	Ron (mRNA)	E7.5- E16.5	Gaudino,G. 1995
Mouse lung	c-ros (mRNA)	E14- adult	Tessarollo,L. 1992 Sonnenberg,E. 1991
Rat lung	T1-alpha	E12.5	Meneghetti,A. 1996
Rat lung	T1-alpha	E13.5-E18	Meneghetti,A. 1996 Rishi,AK. 1995
Rat lung	T1-alpha	E13-E20	Williams,MC. 1996
Rat lung	TGF-beta typeI-R	E18- E21	Yee,W. 1996
Mouse lung	TGF-beta typeII-R	E11 +4days	Zhao,JS. 1996
Rat lung	TGF-beta typeII-R	E18- E21	Yee,W. 1996

Mouse lung	TIA-1 (mRNA)	E12.5 E14.5	Lowin,B. 1996
Mouse lung	TIA-1 (mRNA)	E16.5	Lowin,B. 1996
Mouse lung	TIA-1 (mRNA)	E18.5	Lowin,B. 1996

Expression of Transcription Factors and Nuclear proteins during Lung Development
(http://www.ana.ed.ac.uk/anatomy/database/lungbase/tf-nptb.html)

Species and Organ	Molecule	Stage	Reference
Mouse lung	fkh-6 (mRNA)	E6.5- E12.5	Kaestner,KH. 1996
Mouse lung	c-fos	E14 E17	Molinar-Rode,R. 1993
Rat lung	HDP	E18- E22	Rayani,HH. 1996
Mouse lung	HFH-1 (mRNA)	Adult	Clevidence,DE. 1994
Mouse lung Rat lung	HFH-1 (mRNA)	Adult	Clevidence,DE. 1994
Rat lung	HFH-4/HNF	E14.5 to P0	Hackett,BP. 1995
Mouse lung Rat lung	HFH-4 (mRNA)	Adult	Clevidence,DE. 1994
Mouse lung Rat lung	HFH-8 (mRNA)	Adult	Clevidence,DE. 1994
Mouse lung	HNF3-alpha (mRNA)	E6.5- adult	Monaghan,AP. 1993 Ang,SL. 1993
Mouse lung	HNF3-alpha (mRNA)	Adult	Clevidence,DE. 1994
Mouse lung	HNF3-beta	E6.5- adult	Monaghan,AP. 1993, Ang,SL. 1993, Zhou,L. 1996, Bellusci,S. 1996
Mouse lung	HNF3-beta	E18- adult	Zhou,L. 1996 Clevidence,DE. 1994
Mouse lung	HNF3-gamma (mRNA)	E6.5- adult	Monaghan,AP. 1993
Mouse lung Rat lung	Hox-a5 (mRNA)	E14- adult	Bogue,CW. 1994
Mouse lung	Hox-b1 (mRNA)	E9.5- E12.5	Bogue,CW. 1996
Mouse lung	Hox-b2 (mRNA)	E9.5	Bogue,CW. 1996
Mouse lung	Hox-b2 (mRNA)	E10.5 E12.5	Bogue,CW. 1996
Mouse lung	Hox-b3 (mRNA)	E9.5	Bogue,CW. 1996 Sham,MH. 1992
Mouse lung	Hox-b3 (mRNA)	E10.5 E11.5	Bogue,CW. 1996
Mouse lung	Hox-b3 (mRNA)	E12.5	Bogue,CW. 1996
Mouse lung	Hox-b4 (mRNA	E9.5	Bogue,CW. 1996
Mouse lung	Hox-b4 (mRNA)	E10.5 E12.5	Bogue,CW. 1996
Mouse lung	Hox-b5 (mRNA)	E9.5	Bogue,CW. 1996
Mouse lung	Hox-b5	E10	Bogue,CW. 1996
Mouse lung	Hox-b5	E10 E12.5	Bogue,CW. 1996
Mouse lung	Hox-b5 (mRNA)	E14- adult	Bogue,CW. 1994
Mouse lung	Hox-b6 (mRNA)	E14- adult	Bogue,CW. 1994
Mouse lung	MFH-1	E12.5	Kaestner,KH. 1996
Mouse lung	c-myc (mRNA)	E6.5- P0	Stanton,BR. 1992 Hirning,U. 1991
Mouse lung	L-myc (mRNA)	E9.5- E15.5	Hatton,KS. 1996
Mouse lung	N-myc (mRNA)	E6.5- P0	Stanton,BR. 1992 Hirning,U. 1991
Human lung	Prox-1 (mRNA)	Adult Fetal	Zinovieva,RD. 1991
Mouse lung	tlx-1 (mRNA)	E8.5- E16.5	Raju,K. 1993
Human lung	Topoisomerase II-alpha mRNA	12wk-15wks	Zandvliet,DWJ. 1996
Human lung	Topoisomerase II-beta mRNA	12wk-15wks	Zandvliet,DWJ. 1996
Mouse lung	TTF-1	E10- E16	Zhou,L. Kimura,S. 1996 Lazzaro,D. 1991
Mouse lung	TTF-1	E17- adult	Zhou,L. 1996
Human lung	TTF-1	11wk-22wks	Stahlman,MT. 1996 Ikeda,K. 1995

Human lung	TTF-1	36wk-42wks	Stahlman,MT. 1996 Ikeda,K. 1995

2. Marker Molecules of Mammary Gland

Expression of Cytokines during Mammary Gland Development
(http://www.ana.ed.ac.uk/anatomy/database/mammbase/cytkintb.html)

Species and Organ	Molecule	Stage	Reference
Human mammary gland	Activin-beta-A	Adult	Liu,QY. 1996
Mouse mammary gland	Bmp-2 (mRNA)	E13.5	Phippard,DJ. 1996
Mouse mammary gland	Bmp-4 (mRNA)	E13.5	Phippard,DJ. 1996
Mouse mammary gland	PDGF-A (mRNA)	E14.5	Orr-Urtreger,A. 1992
Mouse mammary gland	Wnt10b (mRNA)	E10.5 Adult	Wang,JW. 1996

Expression of Receptors and Signal Transduction Molecules during Mammary Gland Development
(http://www.ana.ed.ac.uk/anatomy/database/mammbase/recepttb.html)

Species and Organ	Molecule	Stage	Reference
Human mammary gland	ActR-II (mRNA)	Adult	Liu,QY. 1996
Mouse mammary gland	bek (mRNA)	E12.5 E14.5	Orr-Urtreger,A. 1993
Mouse mammary gland	KGFR (mRNA)	E12.5 E14.5	Orr-Urtreger,A. 1993
Mouse mammary gland	PDGFRalpha (mRNA)	E14.5	Orr-Urtreger,A. 1992
Mouse mammary gland	PTHrP/PTH (mRNA)	E14.5	Dunbar,ME. 1996

Expression of Transcription Factors and Nuclear proteins during Mammary Gland Development
(http://www.ana.ed.ac.uk/anatomy/database/mammbase/tf-nptb.html)

Species and Organ	Molecule	Stage	Reference
Mouse mammary gland	LEF-1	E13.5 Adult	Vangenderen,C. 1994
Mouse mammary gland	Msx-1	E13.5	Phippard,DJ. 1996, Friedmann,Y. 1996
Mouse mammary gland	Msx-2	E13.5	Phippard,DJ. 1996
Mouse mammary gland	Msx-2 (mRNA)	Adult	Friedmann,Y. 1996

Distribution of Enzymes, Substrates and misc. molecules. during Mammary Gland Development
(http://www.ana.ed.ac.uk/anatomy/database/mammbase/enz-misc.html)

Species and Organ	Molecule	Stage	Reference
Mouse mammary gland	NEP (mRNA)	E12-15	Weil,M. 1995
Mouse mammary gland	PPT-A (mRNA)	E12-15	Weil,M. 1995

3. Marker molecules of Pancreas Development

Effect of Bioactive Molecules on Pancreatic Cell and Organ Culture
(http://www.ana.ed.ac.uk/anatomy/database/pancbase/culture.html#acta)

Species and Organ	Molecule	Stage	Reference
Human pancreas	Act/Inh beta-A	15-17wks	Turri,T. 1994
Human pancreas	Act/Inh beta-B	15-17wks	Turri,T. 1994
Mouse pancreas	Bmp-7 (mRNA)	E8.5-E14.5	Lyons,KM. 1995
Human pancreas	bFGF (mRNA)	Wk12-Wk16	Gonzalez AM. 1996
Human pancreas	bFGF	Wk12-Wk16	Gonzalez AM. 1996
Mouse pancreas	Glucagon (mRNA)	E7.5-P0	Gittes,GK. 1992 Herrera,PL. 1991
Human pancreas	HGF/SF (mRNA)	18-24wks	Otonkoski,T. 1996

Mouse pancreas	Insulin (mRNA)	E7.5-P0	Gittes,GK. 1992
Mouse pancreas	Insulin I	E8.5-18.5	Herrera,PL. 1991
Mouse pancreas	Insulin II	E8.5-18.5	Herrera,PL. 1991
Mouse pancreas	Pancreatic Polypep.	E7.5-P0	Gittes,GK. 1992
Mouse pancreas	Pancreatic Polypep.	E8.5-18.5	Herrera,PL. 1991
Mouse pancreas	reg-I (mRNA)	E8.5-12	Perfetti,R. 1996
Mouse pancreas	reg-II (mRNA)	E8.5-12	Perfetti,R. 1996
Mouse pancreas	Somatosta-(mRNA)-tin	E7.5-P0	Gittes,GK. 1992 Herrera,PL. 1991

Expression of Receptors and Signal Transduction Molecules during Pancreatic Development
(http://www.ana.ed.ac.uk/anatomy/database/pancbase/recepttb.html)

Species and Organ	Molecule	Stage	Referene
Human pancreas	ActR-II	15-17wks	Turri,T. 1994
Rat pancreas	ActR-IIB (mRNA)	E8-E20	Roberts,VJ. 1994
Human pancreas	ActR-IIB	15-17wks	Turri,T. 1994
Mouse pancreas	ALK3 (mRNA)	E9.5-E15.5	Dewulf,N. 1995
Rat pancreas	bcl-2	E15- adult	Bouwens,L. 1996
Human pancreas	bek (mRNA)	Wk17-Wk18	Partanen,J. 1991
Rat pancreas	CD44 isoforms	E12- adult	Weber,B. 1996
Rat pancreas	CD44s	E12- adult	Weber,B. 1996
Rat pancreas	CD44 variant V6	E12- adult	Weber,B. 1996
Mouse pancreas	Fas (mRNA)	E16.5- adult	French,LE. 1996
Human pancreas	FGFR-1	Wk12-Wk16	Gonzalez AM. 1996 (Partanen,J. 1991)
Human pancreas	FGFR-3 (mRNA)	Wk17-Wk18	Partanen,J. 1991
Rat pancreas (Human pancreas)	FGFR-4	E21	Obergwelsh,C. 1996 (Partanen,J. 1991)
Rat pancreas	Flk-1	E21	Obergwelsh,C. 1996
Human pancreas	hGHR (mRNA)	13.5w-term	Zogopoulos,G. 1996
Mouse pancreas	KGFR (mRNA)	E14.5	Orr-Urtreger,A. 1993
Rat pancreas	c-Kit	E21	Obergwelsh,C. 1996
Human pancreas	c-met/HGFR	18wk-24wks	Otonkoski,T. 1996
Mouse pancreas	PTP-NP (mRNA)	E8.5- adult	Chiang,MK. 1996
Mouse pancreas	TIA-1	E14.5-E18.5	Lowin,B. 1996

Expression of Transcription Factors and Nuclear proteins during Pancreatic Development
(http://www.ana.ed.ac.uk/anatomy/database/pancbase/tf-nptb.html)

Species and Organ	Molecule	Stage	Reference
Mouse pancreas	HNF3-alpha (mRNA)	E6.5- adult	Monaghan,AP. 1993, Ang,SL. 1993
Mouse pancreas	HNF3-beta	E6.5- adult	Zhou,L. 1996, Monaghan,AP. 1993 Ang,SL. 1993
Mouse pancreas	HNF3-gamma (mRNA)	E6.5- adult	Monaghan,AP. 1993
Mouse pancreas	Hox-b3 (mRNA)	E9.5-E12.5	Sham,MH. 1992
Rat pancreas	IDX-1	Adult	Miller,CP. 1994
Mouse pancreas	pdx-1/IPF-1	E8.5- adult	Ohlsson,H. 1993
Mouse pancreas	Prox-1 (mRNA)	E7.5-E18.5	Oliver,G. 1993
Mouse pancreas	Prox-1 (mRNA)	Fetal	Zinovieva,RD. 1991
Rat pancreas	PTF1/p48 (mRNA)	E11.5-E18	Krapp,A. 1996
Mouse pancreas	PTF1/p48 (mRNA)	E14-E16	Krapp,A. 1996
Mouse pancreas	STF-1	E8.5-E17.5	Guz,Y. 1995
Mouse pancreas	tlx-1 (mRNA)	E8.5-E16.5	Raju,K. 1993

Extracellular Matrix and Adhesion Molecules in Pancreatic Development
(http://www.ana.ed.ac.uk/anatomy/database/pancbase/ecm-adh.html)

Species and Organ	Molecule	Stage	Reference
Mouse pancreas	Clusterin (mRNA)	E16.5	French,LE. 1993

		E18.5	
Mouse pancreas	Syndecan-1	E12-E17	David,G. 1993
Mouse pancreas	Syndecan-2	E12-E17	David,G. 1993

Distribution of Enzymes, Substrates and misc. molecules. during Pancreatic Development (http://www.ana.ed.ac.uk/anatomy/database/pancbase/enz-misc.html)

Species and Organ	Molecule	Stage	Reference
Mouse pancreas	Amylase (mRNA)	E7.5-P0	Gittes,GK. 1992
Mouse pancreas	Carboxypeptidase A	E7.5-P0	Gittes,GK. 1992
Mouse pancreas	FasL (mRNA)	E16.5- adult	French,LE. 1996
Rat pancreas	Follistatin (mRNA)	E8-E20	Roberts,VJ. 1994
Mouse pancreas	GLYT1	E14- E18	Jursky,F. 1996
Mouse pancreas	Glucagon (mRNA)	E7.5-P0	Gittes,GK. 1992
Rat pancreas	Keratin K7	Adult	Bouwens,L. 1995
Rat pancreas	Keratin K19	Adult	Bouwens,L. 1995
Rat pancreas	Keratin K20	E15- adult	Bouwens,L. 1996 Bouwens,L. 1995
Rat pancreas	SHBG/ABP	E15-E17	Becchis,M. 1996
Rat pancreas	Vimentin	E17- birth	Bouwens,L. 1996
Rat pancreas	Vimentin	Adult	Bouwens,L. 1996

4. Marker molecule of prostate gland

Expression of Cytokines and Growth Factors during Prostatic Gland Development (http://www.ana.ed.ac.uk/anatomy/database/prosbase/cytkintb.html#act)

Species and Organ	Molecule	Stage	Reference
Rat prostate gland	Activin	Adult	Risbridger,GP. 1996
Rat prostate gland	Inhibin	Adult	Risbridger,GP. 1996

Expression of Transcription Factors and Nuclear Proteins during Prostate Gland Development (http://www.ana.ed.ac.uk/anatomy/database/prosbase/tf-nptb.html)

Species and Organ	Molecule	Stage	Reference
Rat prostate gland (Mouse prostate gland)	Androgen receptor	Adult	Risbridger,GP. 1996 (Cooke,PS. 1991)
Rat prostate gland	Inhibin	Adult	Risbridger,GP. 1996
Rat prostate gland	Estrogen receptor	E13-P0	Cooke,PS. 1991
Rat prostate gland	Fas (mRNA)	Adult	French,LE. 1996

Extracellular Matrix and Adhesion Molecules in Prostatic Gland Development (http://www.ana.ed.ac.uk/anatomy/database/prosbase/ecm-adh.html)

Species and Organ	Molecule	Stage	Reference
Rat prostate gland	Actin (alpha)	E19- adult	Hayward,SW. 1996 Cunha,GR. 1996
Rat prostate gland	Desmin	E19- adult	Hayward,SW. 1996 Cunha,GR. 1996
Mouse prostate gland	FasL (mRNA)	E16.5- adult	French,LE. 1996
Rat prostate gland	Keratin K5	E17- adult	Hayward,SW. 1996
Rat prostate gland	Keratin K7	E17- adult	Hayward,SW. 1996
Rat prostate gland	Keratin K7	E17- adult	Hayward,SW. 1996
Rat prostate gland	Keratin K8	E17- adult	Hayward,SW. 1996
Rat prostate gland	Keratin K14	E17- adult	Hayward,SW. 1996
Rat prostate gland	Keratin K14	E17- adult	Hayward,SW. 1996
Rat prostate gland	Keratin K18	E17- adult	Hayward,SW. 1996
Rat prostate gland	Keratin K19	E17- adult	Hayward,SW. 1996
Rat prostate gland	Myosin	E19- adult	Hayward,SW. 1996 Cunha,GR. 1996
Rat prostate gland	Vimentin	E19- adult	Hayward,SW. 1996 Cunha,GR. 1996
Rat prostate gland	Vinculin	E19- adult	Hayward,SW. 1996 Cunha,GR. 1996

Rat prostate gland	Wnt13 (mRNA)	Fetal Adult	Katoh,M. 1996

5. Marker Molecule of Salivary Gland

Expression of Cytokines during Salivary Gland Development (http://www.ana.ed.ac.uk/anatomy/database/salgbase/cytkintb.html)

Species and Organ	Molecule	Stage	Reference
Mouse salivary gland	Act/Inh beta-A	15, 17, 19dys	Ritvos,O. 1995
Rat salivary gland	Act/Inh beta-A	15, 17, 19dys	Roberts,V.J. 1994
Human salivary gland	Act/Inh beta-A	15-17wks.	Turri,T. 1994
Mouse salivary gland	Act/Inh beta-B	15, 17, 19dys	Ritvos,O. 1995
Rat salivary gland	Act/Inh beta-B	E12-P0	Roberts,V.J. 1994 Roberts,V.J. 1991
Human salivary gland	Act/Inh beta-B	15-17wks.	Turri,T. 1994
Mouse salivary gland	EGF	P12- adult	Gattone,V.H. 1992
Mouse salivary gland	GF	P12-P11	Durban,E.M. 1993
Human salivary gland	GF	15-20wks.	Miettinen,P.J. 1993
Rat salivary gland	EGF	Birth Adult	Martin,M.G. 1990
Human salivary gland	FGF acidic	Adult	Hughes,S.E. 1993
Rat salivary gland	FGF basic	E18	Gonzalez,AM. 1990
Human salivary gland	FGF basic	Adult	Hughes,S.E. 1993
Rat salivary gland	Inhibin- alpha	12-20 days	Roberts,V.J. 1994
Human salivary gland	Inhibin- alpha	15-17 wks.	Turri,T. 1994
Human salivary gland	KL (c-kit ligand)	Adult	Lammie,A. 1994
Mouse salivary gland	N8	18 days	Chen,S.L. 1996
Mouse salivary gland	PDGF-A (mRNA)	E14.5	Orr-Urtreger,A. 1992
Human salivary gland	TGF-alpha	15-20 wks.	Miettinen,P.J. 1993
Mouse salivary gland	TGF-beta-1	E14.5	Heine,U.I. 1987 Lehnert,S.A. 1988
Mouse salivary gland	TGF-beta-2	E12.5	Millan,F.A. 1991
Mouse salivary gland	TGF-beta-2	E14.5	Millan,F.A. 1991
Mouse salivary gland	TGF-beta-3	E9.5- birth	Millan,F.A. 1991

Expression of Receptors and Signal Transduction Molecules during Salivary Gland Development (http://www.ana.ed.ac.uk/anatomy/database/salgbase/tf-nptb.html)

Species and Organ	Molecule	Stage	Reference
Mouse salivary gland	ActR-II	16-20 days	Roberts,V.J. 1994
Human salivary gland	ActR-II	15-17 wks.	Hilden,K. 1994 Turri,T. 1994
Mouse salivary gland	ActR-IIB	13-17 days	Ritvos,O. 1995
Rat salivary gland	ActR-IIB	16-20 days	Roberts,V.J. 1994
Human salivary gland	ActR-IIB	15-17 days	Hilden,K. 1994 Turri,T. 1994
Rat salivary gland	ASGP-R1	1 day	Mu,J.Z. 1993
Mouse salivary gland	bek (mRNA)	E12.5 E14.5	Orr-Urtreger,A. 1993
Rat salivary gland	CD44 isoforms	E12- adult	Weber,B. 1996
Rat salivary gland	CD44s	E12- adult	Weber,B. 1996
Rat salivary gland	CD44 variant V6	E12- adult	Weber,B. 1996
Mouse salivary gland	EGF-receptor	1-20d pp	Durban,E.M. 1995
Mouse salivary gland	EGF-receptor	Birth -P10	Gattone,V.H. 1992
Mouse salivary gland	EGF-receptor	P10- adult	Gattone,V.H. 1992
Human salivary gland	EMA	Adult	Okura,M. 1993
Mouse salivary gland	eps8	E14- E16	Avantaggiato.V. 1995
Mouse salivary gland	eps15	E12.5 E17.5	Avantaggiato.V. 1995
Human salivary gland	FGF-R1	Adult	Hughes,S.E. 1993

Mouse salivary gland	KGFR (mRNA)	E12.5 E14.5	Orr-Urtreger,A. 1993
Human salivary gland	KGFR	Adult	LaRochelle,W.J. 1995
Human salivary gland	c-kit	Adult	Lammie,A. 1994
Mouse salivary gland	PDGFRalpha (mRNA)	E14.5	Orr-Urtreger,A. 1992
Rat salivary gland	c-Ret	Embr.	Tsuzuki,T. 1995
Rat salivary gland	c-Ret	Neon.	Tsuzuki,T. 1995
Rat salivary gland	c-Ret	2-4wk pp	Tsuzuki,T. 1995
Rat salivary gland	c-Ret	7wk pp	Tsuzuki,T. 1995
Mouse salivary gland	TbetaR-I	E12&E13	Colloc. with Iseki,S. 1995
Mouse salivary gland	TbetaR-I	E13&E15	Iseki,S. 1995
Mouse salivary gland	TbetaR-II	E12&E13	Iseki,S. 1995
Mouse salivary gland	TbetaR-II	E13&E15	Iseki,S. 1995
Mouse salivary gland	TIA-1	E16.5 E18.4	Lowin,B. 1996

Expression of Transcription Factors and Nuclear proteins during Salivary Gland Development
(http://www.ana.ed.ac.uk/anatomy/database/salgbase/ecm-adh.html)

Species and Organ	Molecule	Stage	Reference
Mouse salivary gland	BarX1 (mouse Hox homologue)	E13.5-16.5	Tissier-Seta,J.P. 1995
Mouse salivary gland	HNF3-alpha (mRNA)	E6.5- adult	Monaghan,AP. 1993
Mouse salivary gland	HNF3-beta (mRNA)	E6.5- adult	Monaghan,AP. 1993
Mouse salivary gland	HNF3-beta (mRNA)	E6.5- adult	Monaghan,AP. 1993
Rat salivary gland	c-jun	P1 to P14	Lazowski,K.W. 1992
Mouse salivary gland	c-myc	E16.5	Schmidt,P. 1989
Mouse salivary gland	Prothymosin- alpha	18.5d 16.5d	Moll,J. 1996
Mouse salivary gland	Tlx-1 (Hox11)	E8 to E16.5	Raju,K. 1993

Extracellular Matrix and Adhesion Molecules involved in Salivary Gland Development
(http://www.ana.ed.ac.uk/anatomy/database/salgbase/enz-misc.html)

Species and Organ	Molecule	Stage	Reference
Mouse salivary gland	BM-1	E13+48hrs	Hardman,P. 1992
Mouse salivary gland	cea10	Adult	Keck,U. 1995
Mouse salivary gland	Clusterin (mRNA)	E14.5 E18.5	French,LE. 1993
Mouse salivary gland	Collagen type-I	E12 & E13	Nakanishi,Y. 1988
Mouse salivary gland	Collagen type-I	E13 +72hrs	Hardman,P. 1992
Rat salivary gland	Collagen alpha1(I)	0-14d pp	Lazowski,K.W. 1994
Mouse salivary gland	Collagen type-III	E12 early	Nakanishi,Y. 1988
Mouse salivary gland	Collagen type-III	E13 late	Nakanishi,Y. 1988
Mouse salivary gland	Collagen type-IV	E12 & E13	Nakanishi,Y. 1988
Mouse salivary gland	Collagen type-IV	E13+24&48	Hardman,P. 1992
Rat salivary gland	Collagen type-IV	E16 to E18	Kadoya,Y 1989
Rat salivary gland	Collagen alpha1(IV)	0-14d pp	Lazowski,K.W. 1994
Mouse salivary gland	Collagen type-V	E12 & E13	Nakanishi,Y. 1988
Mouse salivary gland	Collagen type-V	E13+24&48	Hardman,P. 1992
Human salivary gland	cra	Adult	Iwai,M. 1991
Mouse salivary gland	Epimorphin	13day	Kadoya,Y 1995
Mouse salivary gland	Epimorphin	17day	Kadoya,Y 1995
Mouse salivary gland	Fibronectin	E13 to 72hrs	Hardman,P. 1992
Mouse salivary gland	Glycosaminoglycans	E13.5	Bernfield,M.R. 1972 Bernfield,M.R. 1982
Mouse salivary gland	Glycosaminoglycans	E13	Cohn,R.H. 1977
Mouse salivary gland	Glycosaminoglycans	E17	Cutler,L.S. 1991
Rat salivary gland	Glycosaminoglycans	E18	Cutler,L.S. 1991
Rat salivary gland	Glycosaminoglycans	E18-P35	Cutler,L.S. 1991
Rat salivary gland	Integrin alpha-1	Adult	Voigt,S. 1994
Human salivary gland	Integrin alpha-2	Adult	Franchi,A. 1994
Human salivary gland	Integrin alpha-5	Adult	Franchi,A. 1994
Mouse salivary gland	Integrin alpha-6	13day	Kadoya,Y 1995
Mouse salivary gland	Integrin alpha-6	E13.5	Kadoya,Y 1993
Mouse salivary gland	Integrin alpha-6	E15	Kadoya,Y 1993
Mouse salivary gland	Integrin alpha-6	E17- adult	Kadoya,Y 1993

			Kadoya,Y 1995
Rat salivary gland	Integrin alpha-6	0-14d pp	Lazowski,K.W. 1994
Mouse salivary gland	Integrin beta-1	13day 17day	Kadoya,Y 1995
Rat salivary gland	Integrin beta-1	0-14d pp	Lazowski,K.W. 1994
Mouse salivary gland	Integrin beta-4	13day	Kadoya,Y 1995
Mouse salivary gland	Integrin beta-4	17day	Kadoya,Y 1995
Mouse salivary gland	Laminin-1	13day	Kadoya,Y 1995
Mouse salivary gland	Laminin	E13.5	Kadoya,Y 1993
Mouse salivary gland	Laminin	E14	Kadoya,Y 1993
Rat salivary gland	Laminin	E15 to E17	Kadoya,Y 1989
Mouse salivary gland	Laminin-1	17day	Kadoya,Y 1995
Mouse salivary gland	Laminin	E13+24&48	Hardman,P. 1992
Mouse salivary gland	Laminin-1	0-14d pp	Lazowski,K.W. 1994
Mouse salivary gland	Laminin alpha-1	13day	Kadoya,Y. 1995
Mouse salivary gland	Laminin alpha-1	17day	Kadoya,Y. 1995
Mouse salivary gland	Laminin 1995 alpha-3A	E17.5	Galliano,MF.
Mouse salivary gland	Laminin 1995 alpha-3B	E13.5	Galliano,MF
Mouse salivary gland	Tenascin-C	13Day	Kadoya,Y. 1995
Mouse salivary gland	Tenascin-C	17Day	Kadoya,Y. 1995

Distribution of Enzymes, Substrates and misc. molecules. during Salivary Gland Development
(http://www.ana.ed.ac.uk/anatomy/database/salgbase/enz-misc.html)

Species and Organ	Molecule	Stage	Reference
Human salivary gland	Actin	Adult	Okura,M. 1993
Human salivary gland	Cytokeratin (gen)	Adult	Draeger,A. 1991 Okura,M. 1993
Human salivary gland	Cytokeratin (gen)	Adult	Zimmer,K.P. 1985
Guinea pig salivary gland	Cytokeratin (gen)	Wk4- birth	Marshak,G. 1987
Guinea pig salivary gland	Cytokeratin 13&16	Wk4- wk6	Marshak,G. 1987
Guinea pig salivary gland	Cytokeratin 13&16	Wk6- birth	Marshak,G. 1987
Human salivary gland	Cytokeratin 19	Adult	Geiger,S. 1987
Mouse salivary gland	Collagenase IV	E13	Reponen,P. 1992
Rat salivary gland	Endopept idase	E18- adult	Dutriez, I. 1992
Mouse salivary gland	FasL	E16.5 E18.5	French,LE. 1996
Mouse salivary gland	Folli-statin	15,17, 19dys	Ritvos,O. 1995
Rat salivary gland	Folli-statin	16-20 days	Roberts,V.J. 1994
Human salivary gland	Folli-statin	15-17 wks.	Turri,T. 1994
Rat salivary gland	Histamine	E10 - adult	Nissinen,M.J. 1995
Rat salivary gland	Histidine Decarboxylase	E10 - adult	Nissinen,M.J. 1995
Mouse salivary gland	Muc-1	E15- E18	Braga,V.M.M. 1992
Mouse salivary gland	Muc-1	E18- adult	Braga,V.M.M. 1992
Rat salivary gland	Peroxidase	21day pp	Redman,R.S. 1993
Mouse salivary gland	pmp22 (mRNA)	E14.5 E16.5	Baechner,D. 1996
Human salivary gland	S100	10-39 wks	Adi,M.M. 1994
Rat salivary gland	SOD (Cu,Zn)	E16 - adult	Munim,A. 1992
Rat salivary gland	SOD (Mn)	E19 - adult	Munim,A. 1992
Human salivary gland	Vimentin	Adult	Okura,M. 1993

Further, the University of California, San Diego, School of Medicine, divided the mice expression stages into the following five stages, and showed it on an online database called the "Kidney Development Gene Expression Database". The list of the genes belonging to each group is shown on the web site (http://organogenesis.ucsd.edu/).

Group1 genes: highest expression early in development

Group2 genes: highest expression in mid-embryogenesis
Group3 genes: highest expression in neonatal life
Group4 genes: increasing though embryogenesis into adulthood
Group5 genes: low expression except in the adult

Each gene is assigned a number, Groups 1 to 5, and it can be seen by this number to which group a certain gene belongs, that is, in which stage of renal development a certain gene expresses. The web pages of each Group are attached for reference.

As stated above, once vertebrates or organs of vertebrates as study objects are specified, the person skilled in the art can determine gene DNA which can be used as stage markers without undue experimentation, and can culture an organ induced from ectoderm region which has been cut off from the blastula of said animal to the same stage as that of the recipient vertebrate, and can transplant the organ into the recipient of same species by ordinary method.

On the contrary, the Examiner has stated in the Action that the previous arguments mentioned above have been fully considered but they are not persuasive for reasons of record and following:

"Even though the principle of basic differentiation such as development, cell and organ differentiation is common to all vertebrates, practicing the claimed invention requires specific, not a sketchy guidance as reiterated by the applicants and cited above. The claims now list genome DNA analysis and cultivation of a particular part of the ectoderm as the inventive step, if these could be done by the knowledge of general principles, they would not be considered as invention.

In practicing step i) of claim 25, it requires precise correlation of distinguishable and unique gene markers correspondent to a particular developmental stage for each genus, subgenus, and species of vertebrates, such markers may not be easily determined by a simple DNA genome search and screening. The developmental gene markers may be known for a few most commonly studies species, they are not known for most of the vertebrates.

In fact, the specification fails to disclose even one developmental stage marker for the species of Xenopus, let alone the entire vertebrate genus."

The Examiner does not understand the present invention correctly. The present invention is not made only by "the knowledge of general principles." The present invention comprises the novel findings according to the present invention, that is, "culturing an organ to the same stage as that of the recipient vertebrate, and transplanting the cultured organ which had been induced in vitro into the recipient vertebrate of same species", and the knowledge of general principles, that is, the content of the Declaration by Professor Makoto Asashima of University of Tokyo that states "in case a particular organ in a particular vertebrate is targeted, the person skilled in the art can easily determine which gene DNA can be used as stage markers by ordinary methods such as the differential display method." Therefore, it is obvious that the Examiner's statement "they would not be considered as invention" does not apply to the present invention.

Further, it can be seen from a number of examples of stage marker gene DNAs as mentioned above that the person skilled in the art can determine which gene DNA can be used as stage markers without undue experimentation. This fact validates the content of the Declaration by Professor Makoto Asashima.

The above-mentioned fact is confirmed by the textbook reference below. That is, in "Series <Applied Animal Science/Bioscience2> Animal Body Formation-Mechanism of Morphogenesis, p. 41" (Hiroyuki Takeda, Asakura Book Co., 2001), it is mentioned that "Genes that exhibit region-specific expression in embryos are easily isolated by the differential screening method, and a number of important genes including Vg1, Chordin, etc., have been already isolated."

In addition, as the ultimate goal of the present invention is mainly the production of human organs for transplantation, it seems good enough for the present invention if developmental gene markers for human and commonly studied species are publicly known. As mentioned above, a number of stage marker gene DNAs of human organs whose stages are specified are known, and it is obvious that the person skilled in the art can determine "precise correlation of distinguishable and unique gene markers correspondent to a particular developmental stage" without undue experimentation.

In the 117th JAMS Symposium, "Stem Cell and Cell Therapy-[I] Stem Cell Biology, 3, "To which extent organ formation is possible with undifferentiated cells". P.27, the present inventor, Professor Makoto Asashima, has stated, "Pronephric tubule is formed by treating undifferentiated cells (animal cap cells) of Xenopus embryo with activin and retinoic acid, and a timecourse analysis of genes after the treatment reveals that the genes express regularly just like a normal embryo. **It is shown that** the genes found by this **method not only express in embryos of frogs and newts, but also involved in the formation of kidney in the early development of mammals such as mouse and human**." This is also described in Development 128, 3105-3115, 2001, and Molecular and Cellular Biology 23, 1, 62-69, 2003.

(B) The Examiner has stated in the Action that the arguments have been fully considered but they do not solve the problems related to the Action because they are not persuasive for the following reason: **when there is no disclosure of any of the conditions under which a process can be carried out, undue experimentation is required; there is a failure to meet the enablement requirement that cannot be rectified by asserting that all the disclosure related to the process is within the skill of the art** (Genentech Inc. v. Novo Nordisk A/S, 42 USPQ2d 1005 (CAFC 1997)) [hereinafter referred to as holding A].

On the other hand, however, The Federal Circuit has stated that: "a specification need not disclose what is well known in the art." See, e. g., HYBRITECH INC. V. MONOCLONAL ANTIBODIES, INC., 802 F.2D 1367, 1385, 231 USPQ 81, 94 (FED. CIR. 1986). [hereinafter referred to as holding B].

In addition, with respect to arguments regarding issued patents having broad claims, the court (*In re Giolito and Hofmann*, 188 USPQ 645 (CCPA 1976)) states "It is immaterial whether similar claims have been allowed to others. See *In re Margaroli*, 50 CCPA 1400, 318 F.2d 348, 138 USPQ 158 (163); *In re Wright*, 45 CCPA 1005, 256 F.2d 583, 118 USPQ 287 (158); *In re Launder*, 41 CCPA 887, 212 F.2D 603, 101 USPQ 391 (1954)." [hereinafter referred to as holding C].

The Examiner has cited many cases. However, if holding A is absolute justice, the decision regarding the holding C must not be given. The fact that the decision

regarding the holding C has been given supports the fact that a patent comprising broad claim(s) is allowed. This is also supported by the holding B. Provided that the present invention comprises broad claims, the Examiner's recognition mentioned in the Action is not persuasive unless the Examiner specifically explains the difference between an allowed broad patent, for example, US patent No. 4237224 (Inventors: Cohen Stanley (Stanford University), Boyer Herbert (University of California)) and the present invention.

(C) In practicing the step ii) of claim 25, the Examiner states, "**The physiological art in general is acknowledged to be unpredictable** (MPEP 2164.03). **It is noted that in applications directed to inventions in arts where the results are unpredictable, the disclosure of a single species usually does not provide an adequate basis to support generic claims.** In re Soll, 97 F. 2d 623, 38 USPQ 189 (CCPA 1938). **In cases involving unpredictable factors, such as most chemical reactions and physiological activity, more may be required**", and believes the rejection stands for reasons set forth below:

"it requires precise knowledge with regard to the type, amount and timing of the growth factors required for proper development for each type of vertebrate, such knowledge may not be predictably extrapolated from Xenopus."

Further, the Examiner has stated as follows with the same references cited in the previous Office Action:

"The molecular basis of the self-renewing pluripotent phenotype remains ill-defined. The relationship between factors that influence embryonic stem cells propagation in vitro and mechanisms of stem cell regulation operative in the embryo is also uncertain" (*Burdon et al*, Cells Tis Org 1999; 165: 131-34);

"Human development is regulated by embryonically and maternally derived growth factors of various kinds at different stages of the embryo development. These growth factors and their receptors would influence the rate of embryo development, the proportion of embryos developing to the blastocyst stage, blastocyst cell number, metabolism and apoptosis by ways of autocrine, paracrine, and endocrine pathways that

may operate within the embryo and between the embryo and the reproductive tract" (*Hardy et al* J Endocrinol 2002; 172: 221-36);

"Even though the method of cloning is common in principle among different vertebrates, the phenotype of cloned vertebrate animals differs significantly, for example, a simple look of the appearance of a xenopus and a sheep would find that they differ in so many ways. Such differences are determined by the genome components as well as cytokines and growth factors during the development. The unpredictability of animal cloning technology lies not on the method steps but the substantial differences in development and resulting phenotypes. Therefore, it is difficult for one skilled artisan to predictably extrapolate from disclosed condition for the development of a xenopus to that of any organ of any vertebrate animal," and

"Although applicants demonstrated that the ectoderm region of Xenopus could differentiation to pronephron under certain conditions, the specification fails to teach whether organs such as heart could also be differentiated from the animal cap, what such conditions are."

The present inventor, Professor Makoto Asashima, has stated that some novel genes such as XCIRP (Uochi T., Asashima M., Gene, 211, 2, 245-250, 1998), XSMP-30 (Sato A., Asashima M., Yokota T., Nishinakamura R., Mech. Dev. 92, 2, 273-275, 2000) and XSal-3, which are candidate genes for gene markers for Xenopus, have been identified by screening genes that express over time in the process of formation of kidney by stimulating the animal cap with activin and retinoic acid. Further, genes such as XCIRP etc. are also described in "The Best Mode for Carrying Out the Invention" in the specification.

Activin is a protein isolated by Vale, W., Ling, N. et al., as a molecule which promotes secretion of follicle-stimulating hormone (FSH) from human anterior lobe of hypophysis (Vale, W. Ling, N. et al., Nature, 321, 776-782, 1986), and belongs to TGF-β family of growth factors. In 1990, the present inventor, Professor Makoto Asashima et al. have found that **activin** isolated from culture supernatant of cultured cell (human K562 cell line) has potent inducing activity to animal caps of newts and Xenopus (Asashima, M. et al., Roux's Arch. Dev. Biol., 198, 330-335, 1990). Further, ensuing

studies have revealed that activin is actually present in the early embryo of Xenopus (T. Ariizumi et al., Proc. Natl Acad Sci USA 1991, 88, 6511-6514), therefore, activin is presumed to be a potent candidate for a mesoderm-inducing factor which functions in an embryo. Although Xenopus embryos were used as cells in the examples described in the present specification, **the activin used** for inducing differentiation **was a human recombinant activin**, therefore **it cannot be considered that the effect of the activin works on Xenopus only and specifically, but it is considered that the effect works on vertebrates in general, including mammals such as human**.

As to the reason why Xenopus embryos are used as development and differentiation-inducing model, only general reasons have been mentioned in many treatises and papers. The reasons are, for example, it is easy to handle and manipulate Xenopus embryos, it is easy to observe their developmental stages, and it is possible to obtain them in quantities. However, many embryologists including the present inventor, Professor Makoto Asashima, use Xenopus embryonic system in experiments because the developmental mechanism of Xenopus embryonic system is applicable to that of other vertebrates.

Further, it has been already reported that BMP-4, which belongs to TGF-β family just like activin, has potent inhibitory activity of neural differentiation in Xenopus embryos (Sasai et al., Nature 1995, 376, 333-336), and there is another report stating that potent inhibitory activity of neural differentiation was observed in a similar experiment using epiblast (undifferentiated ectoderm), an explant of mouse that corresponds to animal cap assay (Kawasaki H. et al., Neuron 2000, 28, 1, 331-340). **As it is suggested that a role BMP-4 plays in a mouse is homologous to a role it plays in Xenopus, it is natural that the person skilled in the art would think that in case where mammalian embryos are stimulated with activin, the same or similar phenomenon which is observed in Xenopus would be observed**.

In addition, there is a report stating that a result of generation of a transgenic mouse overexpressing activin suggested that activin controlled proliferation and differentiation of dermal cells and promoted the recovery of wounds on skin (EMBO, J. 18, 5205-5215, 1999).

Collectively, activin **is presumed to have a same effect not only on amphibian**

but also on mammalian level.

Further, the example in this specification describes that differentiation of pronephric tuble etc. is induced by stimulating the animal cap of Xenopus with various concentrations of activin and retinoic acid. Moreover, the present inventor, Professor Makoto Asashima has succeeded to form pancreas in vitro by time-stagger treatment of the animal cap with high concentration of activin and retinoic acid (Develop. Growth Differ. 42, 175-185, 2000; Develop. Growth Differ. 42, 593-602, 2000).

It is also described that differentiation of heart (Int. J. Biol. 40, 715-718, 1996) and liver (Zoological Science 16, 115-124, 1999) could be induced by treating animal cap with high concentration of activin. As to heart, it is described that all heart-specific gene markers were expressed.

Therefore, according to the above description, it is possible to induce the differentiation of tissues derived from mesoderm such as heart, exemplified in the specification, by treating the animal cap with activin and retinoic acid.

The Written Description Rejection

The Office Action states that claim 24 allegedly lacks written description. The cancellation of this claim renders the rejection moot.

The Indefiniteness Rejection

The Office Action states that claims 18-27 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. The cancellation of claims 18-24 renders the rejection of those claims moot. The Office Action does not provide any reasons why claims 25-27 are allegedly indefinite. Applicants' understanding is that the rejection applies only to cancelled claims 18-24.

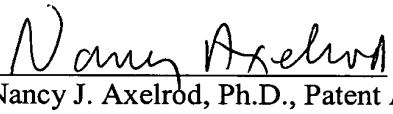
The Anticipation Rejections

The Office Action states that claims 18-22 and 24 are allegedly anticipated, by several references, under 35 U.S.C. §102. The cancellation of those claims renders the rejection moot.

In view of the above amendments are arguments, it is believed the application is in condition for allowance, which action is respectfully requested.

Please charge any additional fee deemed due to Deposit Account No. 22-0261 and advise us accordingly.

Respectfully submitted,



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DC2-DOCS1-442325